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Complete List of Authors:	Ludvigsson, Johnny; Linköping University, Division of Pediatrics, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences; Crown Princess Victoria Children´s Hospital Eriksson, Linnea; Diamyd Medical AB Nowak, Christoph; Diamyd Medical AB; Karolinska Institute, Department of Neurobiology, Care Sciences and Society Teixeira, Pedro F.; Diamyd Medical AB Widman, Martina; Diamyd Medical AB Lindqvist, Anton; Diamyd Medical AB Casas, Rosaura; Linköping University, Division of Pediatrics, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences Lind, M; University of Gothenburg, Department of Molecular and Clinical Medicine; NU-Hospital Group and the Sahlgrenska university Hospital Hannelius, Ulf; Diamyd Medical AB
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A Phase III, Randomized, Double-blind, Placebo-controlled, Multicenter Trial to Evaluate the Efficacy and Safety of rhGAD65 to Preserve Endogenous Beta Cell Function in Adolescents and Adults with Recently Diagnosed Type 1 Diabetes, Carrying the Genetic HLA DR3-DQ2 Haplotype – The DIAGNODE-3 study protocol.

Johnny Ludvigsson[1,2], Linnea Eriksson[3], Christoph Nowak[3,4], Pedro F. Teixeira[3], Martina Widman[3], Anton Lindqvist[3], Rosaura Casas[1], Marcus Lind[5,6,7], Ulf Hannelius[3]

Affiliations:

a) Division of Pediatrics, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden
b) Crown Princess Victoria Children´s Hospital, Linköping, Sweden
c) Diamyd Medical AB, Stockholm, Sweden
d) Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Huddinge, Sweden
e) Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden
f) Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
g) Department of Medicine, NU-Hospital Group, Trollhättan and Uddevalla, Sweden

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Corresponding author:

Professor Johnny Ludvigsson

Division of Pediatrics, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, and Crown Princess Victoria Children's Hospital, Linköping University, SE-58185 Linköping, Sweden

Email: Johnny.Ludvigsson@liu.se

ABSTRACT

Introduction

Type 1 diabetes (T1D) is an autoimmune disease leading to the destruction of the insulin-producing beta cells resulting in insulin deficiency and hyperglycemia. Today, no approved therapy exists to halt this detrimental immunologic process. In a recent Phase 2b study, intralymphatic administration of recombinant human glutamic acid decarboxylase (rhGAD65) adsorbed to Alhydrogel adjuvant to individuals recently diagnosed with T1D and carrying the HLA DR3-DQ2 haplotype showed promising results in preserving endogenous insulin secretion, confirming the results of a large meta-analysis of three randomized placebo-controlled trials of subcutaneous rhGAD65. The aim of the current precision medicine Phase 3 study is to determine whether intralymphatic administration of rhGAD65 preserves insulin secretion and improves glycaemic control in presumed responder individuals with recently diagnosed T1D carrying HLA DR3-DQ2.

Methods and analysis

Individuals ≥12 and <29 years old recently diagnosed with T1D (<6 months) will be screened for the HLA DR3-DQ2 haplotype, endogenous insulin production estimated by fasting C-peptide and presence of GAD65 antibodies. 330 patients are planned to be randomized to three monthly intralymphatic injections of rhGAD65 or placebo (both accompanied by oral Vitamin D supplementation), followed by 22 months of follow-up. The study is powered to detect a treatment effect in the two co-primary endpoints; change from baseline in $AUC_{(0\text{-}120min)}$ C-peptide levels during a mixed meal tolerance test, and change from baseline in glycemic control estimated by HbA1c at 24 months. Secondary endpoints include effects on glucose patterns collected by masked continuous glucose monitoring, proportion of patients in partial remission, and number of episodes of severe hypoglycemia and/or diabetic ketoacidosis.

Ethics and dissemination

The trial will be performed in accordance with ICH-GCP guidelines and principles of the Helsinki Declaration. The study has so far been approved by Ethics Committees in Poland, Netherlands, Sweden, Czech Republic, Germany, and Spain.

Trial registration:

EudraCT identifier: 2021-002731-32

NCT identifier: NCT05018585

Keywords: Type 1 diabetes, immune intervention, rhGAD65, intralymphatic, HLA DR3-DQ2, Phase 3, antigen specific therapy, precision medicine

Article Summary

Strengths and limitations of this study

- The current study is a randomized double-blind placebo-controlled trial. The active treatment in previous rhGAD65 studies has shown minimal treatment-related adverse events and negligible effects on clinical laboratory parameters, making accidental unblinding to investigators very unlikely (which is presumed to have happened in other immune intervention trials).
- The study will investigate the efficacy of treatment on two clinically important co-primary endpoints; preservation of beta cell function and glycaemic control (HbA1c).
- The study is a large, international, multi-centre trial, adequately powered to detect a treatment effect in both co-primary endpoints. The total study duration of 24 months is longer than many previous and ongoing studies in T1D and should allay concerns about confounding from the so-called honeymoon period in recently diagnosed T1D.
- This is the first Phase 3 trial in T1D using a precision medicine approach by only including patients with a specific genetic marker and GAD-autoantibody positivity, limiting recruitment to the previously identified HLA DR3-DQ2 responder population to rhGAD65 treatment.
- However, it is anticipated that to fully understand the magnitude of a possible beneficial treatment effect, additional follow-up over several years might be needed to see the benefits of even minimum residual beta cell function on the prevention of acute and late complications such as severe hypoglycaemia or retinopathy.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disorder in which the immune system attacks the insulin producing beta cells in the pancreas. By the time an individual is diagnosed with T1D, 70-90% of beta cell function has generally been lost. The destruction of the pancreatic beta cells in T1D is associated with cellular immune responses towards the pancreatic islet cells (1). Autoantibodies directed against glutamic acid decarboxylase (GAD) with a molecular mass 65 kDa (GAD65A), insulinoma-associated protein 2 (IA-2A), insulin (IAA), or zinc transporter antigen T8 (ZnT8A) precede the clinical onset of the disease (1).

T1D treatment consists of lifelong administration of exogenous insulin, which does not satisfactorily prevent neither acute nor long-term complications. The disease has a devastating impact on the quality of life (QoL) of the affected person and their family due to the constant stress of adjusting blood sugar and the common acute and life-threatening consequences of imperfect control - diabetic ketoacidosis (DKA) and severe hypoglycaemia (2-4). In addition, many individuals with T1D experience over the long term both macro- and microvascular complications affecting the heart, nerves, eyes and kidneys, putting them at risk of blindness, kidney failure and myocardial ischemia (5, 6). A recent article (7) concluded that patients who received their diagnosis before the age of 10 years had a shortened lifespan by 14 years for males and 18 years for females. Early-onset T1D was also found to be associated with a 30 times increased risk of serious cardiovascular outcomes and for women, this risk was 90 times higher compared to non-diabetic control persons (7). Even with good long-term blood glucose control (HbA1c ≤52 mmol/mol), the risk of premature death for any T1D patient is found to be twice as high as for healthy individuals and up to eight times higher for patients with poor glycemic control (8).

There is currently no approved treatment preventing the destruction of beta cells. Insulin replacement therapy is the standard-of-care treatment, and despite the development of new insulins, new technologies for insulin administration and blood glucose diagnostics, patient targets for long-term blood glucose are currently met less frequently than 5 years ago in some populations in the US (9). Any intervention which can stop or delay the loss of beta cell function would likely provide protection against hypoglycemia and ketoacidosis, improve metabolic control, decrease blood glucose fluctuations, facilitate treatment and delay and/or reduce micro and macrovascular complications of diabetes (10-13). Additionally, decreasing the autoimmune destruction of beta cells could allow for beta cell replenishment, either through regeneration or transplantation.

The most efficient immune therapy for preservation of beta cell function is so far treatment with anti-CD3 monoclonal antibodies (teplizumab) (14, 15). TNF-alfa inhibitors (16), anti-thymocyte globulin (ATG) (17), alefacept (18) and rituximab (19) have also demonstrated some efficacy in preserving beta cell function, but often these therapies have adverse events, serious risks and impose a heavy treatment burden, including e.g. several days of intravenous infusions. An alternative approach is treatment with autoantigen immunotherapies, even though most clinical trials with autoantigen immunotherapies have failed to meet their primary endpoints or shown inconclusive results (20-23).

Over the last two decades, however, an important development in the field has meant a shift away from a one-size-fits-all approach to T1D pathophysiology towards a more individualized, precision medicine approach that recognizes inter-individual heterogeneity in T1D (24). The concept of disease heterogeneity has recently been extended to the concept of endotypes; that is, subtypes of T1D with distinct underlying pathobiological mechanisms, which should be considered in the design of clinical trials (25). For instance, the appearance of GAD65 autoantibodies (GADA) as the first autoantibody is linked to the Human Leukocyte Antigen (HLA) DR3-DQ2 haplotype, while emergence of insulin autoantibodies as the first antibody is linked to HLA DR4-DQ8 (25, 26). As a consequence, applying the same intervention targeting a specific pathophysiologic mechanism across an entire population ignores the fact that subgroups of patients whose disease is driven by the targeted mechanism may respond particularly well, whilst others show no response, resulting in apparently absent treatment effects across the entire population.

GAD antigen-specific immunotherapy to preserve endogenous insulin secretion

GAD65 is a major autoantigen in autoimmune diabetes and clinical administration of purified recombinant human GAD65 rhGAD65 aims to intervene in the autoimmune process in T1D. The rhGAD65 is adsorbed to Alhydrogel® (aluminium hydroxide particles) and formulated in phosphate buffer with mannitol. The intended mode of action is to slow or prevent autoimmune destruction of pancreatic beta cells by modulation of immune responses to GAD65. Inconsistent results observed in trials testing subcutaneous administration of rhGAD65 spurred the evaluation of alternative approach to improve treatment efficacy (20-22, 27). DIAGNODE-1 (28, 29), a Phase 1/2a open-label pilot combination trial evaluated an alternative administration route, with three doses of 4 µg rhGAD65 administered directly into inguinal lymph nodes, in combination with oral Vitamin D in 12 patients (12 to 30 years of age) recently diagnosed with T1D. All patients were followed for 30 months. The positive results of the DIAGNODE-1 trial

(29) supported further development in a Phase 2b trial (DIAGNODE-2), a randomized and placebo-controlled trial testing the intralymphatic administration in 109 patients (12 to 24 years of age) recently diagnosed with T1D. Importantly, based on the concept of heterogeneity of disease mechanism, a meta-analysis of three previous RCTs testing subcutaneous rhGAD65 was performed. The analysis showed that clinical efficacy is mainly seen in patients with HLA DR3-DQ2, and an even more pronounced treatment effect was seen in those individuals with HLA DR3DQ2 without HLA DR4-DQ8 though no clinical efficacy was observed in the full population(30). Due to the identification of HLA DR3-DQ2 patients as the responder population, the statistical analysis plan of the then on-going DIAGNODE-2 study was amended before database lock to include analyses of the primary and secondary endpoints in the HLA DR3-DQ2 subgroup in the topline results. At 15 months of follow up in DIAGNODE-2, rhGAD65 treatment showed a significant positive treatment effect in the pre-specified genetic subgroup of patients positive for HLA DR3-DQ2 of 55.7% (p-value 0.0078), i.e. that on average, the primary end point stimulated C-peptide secretion $AUC_{mean\ 0\text{-}120\ min}$ declined by 55.7% less in patients treated with rhGAD65 compared to patients treated with placebo. For patients positive for HLA DR3-DQ2, C-peptide $AUC_{mean\ 0\text{-}120\ min}$ declined approximately 28% over 15 months in the rhGAD65 group compared to about 58% for placebo (31). There were corresponding trends, though not statistically significant, in improvement in the secondary efficacy variables HbA1c, IDAA1c and exogenous insulin use after 15 months in the HLA DR3-DQ2-positive patients treated with rhGAD65 when compared to placebo.

Results from an updated meta-analysis (manuscript in preparation) which added data from DIAGNODE-2 to the previous meta-analysis (30) showed that in patients carrying the HLA DR3-DQ2-haplotype, a statistically significant treatment effect on change in AUC C-peptide of 48.3% for the subjects receiving 3 or 4 injections of rhGAD65 ($p < 0.0001$) and 36.1 % for the 2-4 injections ($p = 0.0316$) . In addition to this, a statistically significant treatment effect on change in HbA1c of -4.789 mmol/mol for the subjects receiving 3 or 4 injections of rhGAD65 ($p = 0.0044$) and -3.120 mol/mol for subjects receiving 2-4 injections ($p = 0.032$). The data also reconfirmed previous findings that an even more pronounced treatment effect (on both change in AUC C-peptide and HbA1c) was seen in those individuals with HLA DR3DQ2 without HLA DR4-DQ8. Intralymphatic rhGAD65 injections were well tolerated and considered safe, consistent with prior clinical trial findings (31).

Objectives

The primary objective is to evaluate the effect of three doses of rhGAD65 compared to placebo in terms of (1) beta cell function; and (2) glycemic control in adolescents and adults recently diagnosed with T1D, who carry the HLA DR3-DQ2 haplotype. Secondary objectives are to compare the effect of rhGAD65 to placebo treatment with respect to the effects on other diabetes disease management indicators and long-term safety.

METHOD AND ANALYSIS

Overall Study Design

DIAGNODE-3 is a Phase III randomized, double-blind, placebo-controlled, international, multicenter, parallel-arm, 24-month trial in adolescents and adults with recently diagnosed T1D, carrying the HLA DR3-DQ2 haplotype. Overall study design is shown in Figure 1. The study is registered on Clinicaltrials.gov (NCT05018585). The study is expected to take 4.5 years to complete. This includes an intervention with follow-up for 24 months.

Throughout the study duration, all patients will receive standard-of-care routine treatment for their diabetes according to ADA guidelines (amended as appropriate to reflect local standard of care).

Screening and Run-In Period

Patients deemed eligible and/or their parent(s)/legal guardian(s) will have the study explained to them and will receive written patient information. After having had the time to review the study, they will have the opportunity to ask questions to the investigational team. After this, if the patient agrees to participate, they will sign and date the written informed consent form and for patients who are minors, both age-appropriate assent (according to local regulations) and parent's/caregiver's consent is collected. Patients and their parent(s)/legal guardian(s), when applicable, will provide written informed consent before any study-related procedures are performed. The patient and/or their parent(s)/legal guardian(s) will then receive a copy of the signed and dated patient information. Detailed study assessments are shown in Table 1. HLA genotyping of the patient is performed at the first Screening visit after preliminary eligibility is confirmed. If the patient is carrying the HLA DR3-DQ2 haplotype, the patient will attend the second Screening visit (Visit 1B) to perform the remaining Screening procedures. After Screening, patients deemed eligible will proceed to the Run-In period (beginning at Visit 1C) undergo masked CGM for 14 days, receive diabetes education and collect self-reported diabetes information in their eDiary.

Patients with a Screening Vitamin D level <100 nmol/L (40 ng/mL) will start oral Vitamin D supplementation (2000 IU daily) beginning at Visit 1C, 30 days prior to randomization. During the period of supplementation, Vitamin D should be discontinued temporarily if the level exceeds 125 nmol/L (50 ng/mL) and may be resumed when levels are <100 nmol/L (40 ng/mL).

TABLE 1 SCHEDULE OF ASSESSMENTS IN DIAGNODE-3

	Screening		Run-In	Double-Blind Treatment Period			Double-Blind Follow-Up Period								
VISIT	**1A**	**1B**	**1C**	**2 (Baseline)**	**3**	**4**	**5** ☎	**6**	**7**	**8**	**9**	**10** ☎	**11 EoS**	**ET**	**UNS Visit**
Trial Day	**-55 to -40**	**-40**	**-30**	**0**	**30**	**60**	**90**	**180**	**360**	**450**	**540**	**630**	**720**		
Trial Month	**-2**		**-1**	**1**	**1**	**2**	**3**	**6**	**12**	**15**	**18**	**21**	**24**		
Visit Window (days)		**±5**	**±5**	**0**	**±5[l]**	**±5[l]**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**		
Informed consent	X														
Informed consent for genomic sub-study									X						
Review eligibility criteria	X	X	X	X											
Demographic information	X														
Medical history	X	X													
Family history of T1D	X														
Examinations															
Physical examination		X complete		X limited	X limited	X limited		X limited	X limited	X complete	X limited		X complete	X complete	X limited
Neurological assessment		X		X	X	X		X	X	X	X		X	X	X
Tanner staging (if <18 years)				X				X	X		X		X		
Vital signs		X	X	X	X	X		X	X	X	X		X	X	X
Height and weight		X		X	X	X		X	X	X	X		X	X	X
Trial Activities															
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diabetes education			X			X			X		X				
Adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X
Vitamin D supplementation if <100 nmol/L (40 ng/mL)			X Start	X	X	X	X End								
MMTT[j]				X				X		X			X		
Continuous glucose monitoring (blinded)			X					X		X			X		
Randomization				X											
Intralymphatic study drug administration				X	X	X									
Injection site inspection, site staff				X	X	X									
Monitoring of target and adjacent lymph nodes using ultrasound				X	X	X									
eDiary															
Distribution of eDiary			X												
Review of eDiary				X	X	X	X	X	X	X	X	X	X	X	X
Glucose events collection in eDiary			X	X	X	X	X	X	X	X	X	X	X		X

	Screening		Run-In	Double-Blind Treatment Period			Double-Blind Follow-Up Period								
VISIT	**1A**	**1B**	**1C**	**2 (Baseline)**	**3**	**4**	**5** ☎	**6**	**7**	**8**	**9**	**10** ☎	**11 EoS**	**ET**	**UNS Visit**
Trial Day	**-55 to -40**	**-40**	**-30**	**0**	**30**	**60**	**90**	**180**	**360**	**450**	**540**	**630**	**720**		
Trial Month	**-2**		**-1**	**1**	**1**	**2**	**3**	**6**	**12**	**15**	**18**	**21**	**24**		
Visit Window (days)		**±5**	**±5**	**0**	**±5[l]**	**±5[l]**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**		
Review of injection site inspection, eDiary					X	X	X								
Patient-reported activities in eDiary			X					X		X			X		
Daily insulin collection in eDiary			X	X	X	X	X	X	X	X	X	X	X		
Blood Samples for Diabetes Status															
Fasting C-peptide		X		X	X	X		X	X	X	X		X	X	
MMTT-induced C-peptide				X				X		X			X		
MMTT-induced glucose				X				X		X			X		
HbA1c		X		X	X	X		X	X	X	X		X	X	
Fasting plasma glucose		X		X	X	X		X	X	X	X		X	X	
Blood and Urine Sampling for Safety, Genetics, Vitamin D Levels and Immunology															
Hematology		X		X				X	X	X	X		X	X	
Clinical chemistry		X		X	X	X		X	X	X	X		X	X	
Lipid panel (fasting)				X						X			X		
TSH (reflex Free T4), TPO		X								X			X		
Transglutaminase antibody titer				X						X			X		
IA-2 antibody titer				X						X			X		
Vitamin D[s]		X		X	X	X		X	X	X	X		X		
Pregnancy testing, (FOCBP only)		X serum		X urine	X urine	X urine		X urine	X urine	X urine	X urine		X urine		
FSH, LH		X		X		X				X	X		X		
Microalbuminuria (UACR)				X				X	X	X	X		X		
GAD65A titer		X		X	X	X		X	X	X	X		X	X	
HLA class II genotyping	X														
COVID-19 antibody testing				X						X			X		
Other immunologic parameters				X	X	X		X		X			X		
DNA sample collection for genomic sub-study									X						
Quality of Life Assessments															
PedsQL questionnaires				X				X		X			X		

Abbreviations: AE = adverse event; BP = blood pressure; CGM = continuous glucose monitoring; COVID-19 = Coronavirus Disease 2019; DKA = diabetic ketoacidosis; DNA = deoxyribonucleic acid; eCRF = electronic case report form; eDiary = electronic diary; EoS = end of study; ET = early termination; FOCBP = females of childbearing potential; FSH = follicle stimulating hormone; GAD65A = glutamic acid decarboxylase with molecular mass 65 kDa antibody; HbA1c = hemoglobin A1c; HCG = human chorionic gonadotropin; HLA = human leukocyte antigen; HR = heart rate; IA-2 = insulinoma-associated protein 2; ICF = informed consent form; LH = luteinizing hormone; MMTT = mixed meal tolerance test; T1D = type 1 diabetes; T4 = thyroxine; TPO = thyroid peroxidase antibody; TSH = thyroid stimulating hormone; UACR = urine albumin to creatinine ratio; UNS = unscheduled.

Double-Blind Treatment Period and Long Term Follow up.

At Visit 2, patients will be randomized 2:1 to one of the following two treatment groups:

Treatment Group 1:	3 intralymphatic injections of 4 µg (0.1 mL) of rhGAD65 administered on Days 0, 30 and 60
Treatment Group 2:	3 intralymphatic injections of 0.1 mL placebo administered on Days 0, 30 and 60

Randomization will be performed by an Interactive Web Response Systems and stratified by HLA subgroup (presence or absence of HLA DR4-DQ8) and by region. The maximum number of adults (>18 years old) recruited into the trial is 160. rhGAD65 or placebo injections will be administered in the inguinal lymph node by qualified personnel with the help of ultrasound. Vitamin D levels will be monitored throughout the trial. Vitamin D oral supplementation (2000 IU daily) will be administered from Day -30 (Visit 1C) through Day 90 for a total of 120 days for patients with a level <100 nmol/L (40 ng/mL) at Screening. All patients will continue to receive intensive insulin therapy via multiple daily injections or via CSII. Safety will be assessed via physical examinations, neurological assessments, vital signs, clinical laboratory assessments, injection site reactions, and AEs. After the double blinded treatment period of 2 months, patients will be followed in a blinded manner for 22 months. An independent DSMB will be appointed to review unblinded safety data (at least twice a year).

Study Population

Individuals between ≥12 and <29 years old, will be eligible for enrollment if they have been diagnosed with T1D within the previous 6 months at the time of screening, positive for the HLA DR3-DQ2 haplotype, fasting C-peptide is ≥0.12 nmol/L (0.36 ng/mL) and seropositive for GADA. Full list of inclusion and exclusion criteria is shown in Table 2 and Table 3.

TABLE 2 - INCLUSION CRITERIA IN THE DIAGNODE-3 STUDY.

Patients are eligible to be included in this study if all the following criteria apply:	
1.	Must be capable of providing written, signed, and dated informed consent; and for patients who are minors, age-appropriate assent (performed according to local regulations) and parent/caregiver consent.
2.	Males and females aged ≥12 and <29 years old at the time of Screening.
3	Diagnosed with T1D (according to the American Diabetes Association [ADA] classification) ≤6 months at the time of Screening.
4	Possess the HLA DR3-DQ2 haplotype (all patients will be tested; prior genetic testing results will not be accepted).
5	Fasting C-peptide ≥0.12 nmol/L (≥0.36 ng/mL) on at least one occasion (maximum two tests on different days during the Screening period).
6	Possess detectable circulating GAD65 antibodies (lowest level of detection defined by the method used by the central laboratory).
7	Possess HbA1c levels between 35 to 80 mmol/mol (5.4 to 9.5%) on at least one occasion prior to randomization (maximum one additional test within one month from Visit 1B).
8	Be on a stable insulin dose or insulin dosing regimen for one month prior to inclusion with limited fluctuation of daily insulin requirement based on investigator's assessment. For example, if the average insulin dose/kg/24h over a 7-day period compared to the previous 7-day period does not vary more than approximately 15% and/or if the daily insulin dose does not vary more than 0.1 U/kg/24h, the dose can be considered stable. Individuals that are diagnosed with T1D according to the ADA classification but are not taking insulin are eligible to participate.
9	i) Females of childbearing potential (FOCBP) must agree to avoid pregnancy and have a negative pregnancy test performed at the required study visits. FOCBP must agree to use highly effective contraception, during treatment and, until 90 days after the last administration of study medication. ii) Male patients must agree to remain abstinent from heterosexual sex during treatment and for 90 days after treatment or, if sexually active, to use two effective methods of birth control (e.g., male uses a condom and female uses contraception) during and for 90 days after treatment.

TABLE 3 - EXCLUSION CRITERIA IN THE DIAGNODE-3 STUDY

Patients are not eligible to be included in this study if any of the following criteria apply:	
1	Participation in any other trial aimed to influence beta cell function from time of diagnosis of T1D.
2.	Treatment with any oral or non-insulin injectable anti-diabetic medication within 3 months prior to Screening.
3.	History of maturity-onset diabetes of the young (MODY).
4.	Pancreatic surgery, chronic pancreatitis, or other pancreatic disorders that could result in decreased beta cell capacity (e.g., pancreatogenous diabetes).
5.	History of DKA or severe hypoglycemia requiring hospitalization within one month before Screening, or severe episodes of hypoglycemia requiring third party assistance within one month before Screening.
6.	Signs or symptoms suggesting very poorly controlled diabetes e.g., ongoing weight loss, polyuria or polydipsia.
7.	Hematologic condition that would make HbA1c uninterpretable including: a) Hemoglobinopathy, with the exception of sickle cell trait or thalassemia minor; or chronic or recurrent hemolysis. b) Donation of blood or blood products to a blood bank, blood transfusion or participation in a clinical study requiring withdrawal of >400 mL of blood during the 8 weeks prior to the Screening visit. c) Significant iron deficiency anemia. d) Heart malformations or vaso-occlusive crisis (VOC) leading to increased turnover of erythrocytes.
8.	Treatment with marketed or over-the-counter Vitamin D at the time of Screening and unwilling to abstain from such medication during the 120 days when the patient will be supplemented with the study-provided Vitamin D. A patient currently taking Vitamin D at the time of Screening must be willing to switch to the study-provided Vitamin D treatment and to administer it per the study requirements.
9.	Any clinically significant history of an acute reaction to a vaccine or its constituents (e.g., Alhydrogel).
10.	Treatment with any (live or inactive) vaccine, including influenza vaccine and Coronavirus Disease 2019 (COVID-19) vaccine, within 4 weeks prior to planned first study dose of study drug; or planned treatment with any vaccine up to 4 weeks after the last injection with study drug.
11.	Any acute or chronic skin infection or condition that would preclude intralymphatic injection.
12.	Recent (past 12 months) or current treatment with immunosuppressant therapy, including chronic use of glucocorticoid therapy. Inhaled, topical, and intranasal steroid use is acceptable. Short courses (e.g., ≤5 days) of oral or intra-articular injections of steroids will be permitted on trial.
13.	Continuous/chronic treatment with prescribed or over-the-counter anti-inflammatory therapies. Short-term use (e.g., <7 days) is permissible, for example to treat a headache or in connection with a fever.
14.	Known or suspected acute infection, including COVID-19 or influenza, at the time of Screening or within 2 weeks prior to Screening. After confirmed recent COVID-19 infection, a negative polymerase chain reaction test will be required before randomization.
15.	A history of epilepsy, head trauma or cerebrovascular accident, or clinical features of continuous motor unit activity in proximal muscles.
16.	Known diagnosis of human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection. Patients with previous hepatitis C infection that is now cured may be eligible.
17.	Any clinically significant concomitant medical condition
18.	History of significant hepatic disease
19.	Estimated glomerular filtration rate (eGFR) calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) for those >18 years old, and by the Schwartz equation for those 12 to 18 years old, <90 mL/min per 1.73 m or rapidly progressing renal disease.
20.	Patients with hypothyroidism or hyperthyroidism must be on stable treatment for at least 3 months prior to Screening (with normal free thyroxine [T4] levels if hypothyroid).
21.	Any clinically significant abnormal findings during Screening, and any other medical condition(s) or laboratory findings that, in the opinion of the investigator, might jeopardize the patient's safety or ability to complete the trial.
22.	History of malignancy not in remission within the last 5 years other than adequately treated basal cell or squamous cell skin cancer or cervical carcinoma in situ.
23.	Patients with any mental condition rendering him/her unable to understand the nature, scope and possible consequences of the trial, and/or evidence of poor compliance with medical instructions at Screening or showing non-compliance during the Run-In Period.
24.	A history of alcohol or drug abuse or dependence within the past 12 months based on DSM IV criteria.
25.	Current or previous participation in a trial of Diamyd.
26.	Participation in a clinical trial involving administration of an investigational drug in the past 3 months or 5 half-lives (whichever is longer) prior to first dosing of study drug or during the trial.
27.	Females who are breastfeeding, pregnant or plan to become pregnant during the trial.
28.	Patients who in the opinion of the investigator will not be able to follow instructions and/or follow the study procedures or patients that are unwilling or unable to comply with the provisions of this protocol.
29.	An employee or immediate family member of an employee of Diamyd Medical AB.

Study Assessments

Demographics and study procedures

Demographics, baseline data medical history and family history of T1D will be collected at screening. Medical examinations (i.e physical, neurological, and vital signs) will be performed at all on site visits. Patients will also be provided with an eDiary to collect self-reported data on daily insulin dose, injection site reactions, significant glucose events (mild/moderate/severe hypoglycaemic events and DKAs), as well as mealtimes and physical activity. Patients and caregivers (if applicable) will answer the Pediatric Quality of Life Inventory (PedsQL) questionnaires at four visits between baseline and Month 24 to assess Quality of Life. Timings of all assessment can be found in Table 1.

Clinical Laboratory assessments

Laboratory assessments of diabetes status

The timing of all study assessments is presented in Table 1 All laboratory parameters will be analyzed at a central laboratory. A 2-hour MMTT following an overnight fast (>10 hours) will be performed at baseline and at month 6, month 15 and month 24. Meal stimulated plasma glucose and C-peptide levels will be assessed throughout the MMTT (32).

Patients should come to the study site following an overnight fast (>10 hours) and have a plasma glucose level between 3.5 to 12 mmol/L (63 to 216 mg/dL) at home in the morning. Patients are allowed to take basal-insulin day/night before, but not in the morning before the MMTT. Patients should also not administer any short/direct acting insulin within 6 hours before the MMTT. Patients with CSII (insulin pump) must continue with their basal dose insulin, but not add any bolus dose during the last 6 hours before the MMTT.

Samples for HbA1c will be analyzed at a National Glycohemoglobin Standardization Program (NGSP) certified central laboratory. Results will be reported in both International Federation of Clinical Chemistry (IFCC) (mmol HbA1c/mol Hb) and NGSP (% HbA1c) units. Serum samples for fasting glucose and fasting C-peptide levels will also be collected and analyzed throughout the trial.

Safety and other laboratory assessments

All patients will undergo HLA class II genotyping to assess the presence of HLA haplotypes DR3-DQ2 and DR4-DQ8 during the screening procedure. Samples will also be collected for clinical chemistry, hematology, urinalysis, lipids (total cholesterol, LDL-C, HDL-C, and

triglycerides), Vitamin D, Thyroid stimulating hormone, Thyroid peroxidase antibody, Transglutaminase antibody, IA-2 antibody, GAD65 antibody, SARS-CoV-2 antibody, and for females; human chorionic gonadotropin, follicle stimulating hormone and luteinizing hormone. Timing of the assessments can be found in Table 1 and all samples will be analyzed at a central laboratory.

Immunology Assessments

Timing of immunological assessments is indicated in Table 1. GAD65 antibody titers will be measured at a central laboratory. Additional variables to evaluate the influence of treatment on the immune system include GAD65 antibody isotypes, IA-2 antibodies, and cell mediated immune response by proliferation and cytokine secretion upon GAD65 stimulation of PBMC.

Continuous Glucose Monitoring

CGM will be performed for 14 days during the Run-In Period (following Visit 1C) and at three other timepoints during the trial. The timing of the distribution of the glucose monitoring system and assessments is presented in Table 1. The FreeStyle Libre Pro/Pro iQ devices are intended for use only by healthcare professionals, with the patients being blinded to the CGM sensor readings. The devices will be used for data collection during the clinical trial but not to inform decisions on diabetes management and therapy adjustments. Patients will be allowed to use an unblinded CGM device to manage their diabetes and adjust the therapy based on the glucose levels registered.

Time Period and Frequency for Collecting AE and SAE Information

Any worsening in the patient's condition after administration of study drug and up to the end of study or early termination visit should be considered an AE. All AEs will be collected throughout the whole study period (starting from Visit 1C), reviewed and assessed for causality by the investigators at the time points specified in Table 1. Injection site reactions will be collected during the 7 days following study drug injections (Visits 2, 3 and 4), starting the day after the injection. Injection site reactions persisting after 7 days should be reported as an AE.

Statistical considerations

Sample Size and Power

The primary efficacy analysis will be performed in the Full Analysis Set (FAS). The primary efficacy variables will be (1) change from baseline to Month 24 in log-transformed C-peptide $AUC_{mean\ 0\text{-}120\ min}$ during an MMTT and (2) change from baseline to Month 24 in mean HbA1c. The co-primary endpoints will be tested in both the overall population and in the subgroup of patients who carry the HLA DR3-DQ2 haplotype and simultaneously do not carry the DR4-DQ8 haplotype (hereafter the HLA DR4-DQ8-negative subgroup). The overall two-sided 5% Type I error rate will be controlled using a fallback procedure. A two-sided 4% alpha will be assigned to the primary efficacy analysis in the overall population. The remaining 1% alpha will be assigned to the primary efficacy analysis in the HLA DR4-DQ8-negative subgroup

A total sample size of 288 patients is planned for a 2:1 randomization to the rhGAD65 and placebo arms, respectively. This achieves 90% power to detect a clinically relevant difference of 40% in geometric mean ratio C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at Month 24 between the rhGAD65 arm and placebo arm using a two-sided test at the 4% significance level. This is based upon a t-test employing natural log transformation of C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at Month 24 and assumed CV of 0.95 based on simulations of EoS (Month 15) results in the placebo group of the Phase 2b study DIAGNODE-2. Allowing for 12% drop out to Month 24, approximately 330 patients will be randomized.

Statistical Analyses

Primary Efficacy Analysis

Change from baseline in the co-primary endpoints will be analyzed using a Restricted Maximum Likelihood-based repeated measures approach (MMRM). The model for analysis will include fixed, categorical effects of such as for treatment, and stratification variables, as well as interactions effects such as between baseline value-by-visit, and the fixed continuous covariates such as baseline age. Patient identification number will be included as a categorical random effect. An unstructured covariance matrix will be assumed. If this analysis fails to converge, compound symmetry will be tested. The (co)variance structure converging to the best fit, as determined by Akaike's information criterion will be used as the primary analysis. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom. Additional methods of sensitivity analyses may be performed in the event that the proportion of missing values is greater than 5%. These analyses will compare the results from the MMRM model, which assumes that values are missing at random (MAR), with analyses assuming the values are missing not at random (MNAR) such as: return-to-baseline using multiple imputation and tipping point analysis. All sensitivity analyses will be regarded as exploratory, thus no formal adjustment for multiplicity will be performed.

Negligible measurement error is expected, which is assumed to affect all patients, time points and treatment groups equally. The total variance used in the sample size calculation is constructed from the within-subject and the between-subject component of variation. Based on the assumption that the measurement error is the same for everyone, it is therefore accounted for in the total variance estimate.

The co-primary endpoints will be tested sequentially meaning that C-peptide is tested first, and, if significant, HbA1c is tested. Both co-primary endpoints need to meet the statistical significance criterion. The fallback procedure described by Wiens and Dmitrienko (33) will be used to test the primary endpoints in the overall population and in the HLA DR4-DQ8-negative subgroup.

If either of the co-primary endpoints is not statistically significant in the overall population at a two-sided significance level of 0.04, the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.01 level of significance in an analogous manner to the primary analysis in the overall population. If both co-primary endpoints in the overall population are statistically significant at the two-sided 0.04 level, then the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.05 level of significance in an analogous manner to the primary analysis in the overall population. The analysis of secondary and exploratory endpoints will be described in a statistical analysis plan (see appendix) which will be finalized before the first patient is enrolled.

Conclusion

T1D is an autoimmune disease leading to the destruction of beta cells and insulin deficiency, yet no approved therapy exists to halt this detrimental process. Preserving residual beta cell function facilitates insulin treatment and optimization of the glucose control as well as significantly decreasing the risk of both acute and long-term complications positively affecting quality of life and preventing large healthcare costs for society. In conclusion, we expect that the current study will determine whether treatment with rhGAD65 injections in inguinal lymph nodes leads to preserved beta cell function with good glycaemic control among persons newly diagnosed with Type 1 diabetes who carry the HLA DR3-DQ2 haplotype.

Patient and public involvement

Patients were not involved in the study design. Patients and Patient organizations (in Sweden Barndiabetesfonden) support recruitment through dissemination of information and participation in press conferences. Participating patients and caregivers will be informed about the outcome of the trial via webcast, letter and personal communication on the completion of the trial.

ETHICS APPROVAL

The trial will be performed in accordance with International Council for Harmonisation (ICH) guidelines, Good Clinical Practice (GCP) and principles of the Helsinki Declaration. The study has been approved by Ethics Committees in Poland (ref number: 124/2021), Netherlands (ref number: R21.089), Sweden (Ref number: 2021-05063), Czech Republic (ref number: EK-1144/21) Germany (ref number: 2021361) and Spain (ref number: 21/2021). Recruitment of participants is planned to start during 2022. Once the trial is completed, results will be published in international peer-reviewed scientific journals and presented at national and international conferences.

Contributorship statement

JL conceived the idea and wrote the protocol for the DIAGNODE-1 trial on which the current trial is based on. Thus, the design of DIAGNODE-3 is based on the ideas of JL, with further support from UH, MW and AL. The protocol is written by JL, UH, LE, CN, PFT, MW, AL. JL, UH, LE, CN, PFT, MW, AL, RC and ML have taken part in writing and reviewing the manuscript. All authors have approved the manuscript for publication.

Competing Interest

JL has received unrestricted grants from Diamyd Medical, and honoraria as consultant from Dompé International and Provention Bio. ML has received research grants from Eli Lilly and NovoNordisk and been a consultant or received honoraria from Astra Zeneca, Boehringer Ingelheim, Eli Lilly and NovoNordisk. LE, CN, PFT, MW, AL and UH are all employees of Diamyd Medical. CN, PFT, MW and UH own shares in Diamyd Medical.

Funding

This trial is sponsored by Diamyd Medical AB. This protocol is based on previous protocols initially used in DIAGNODE-1 and DIAGNODE-2, studies supported by Barndiabetesfonden (The Swedish Child Diabetes Foundation), Diabetesfonden (the Swedish Diabetes Association), FORSS (Research Council of Southeast Sweden) and ALF/County Council Region Östergötland.

Data sharing statement

No additional data available

Licence statement

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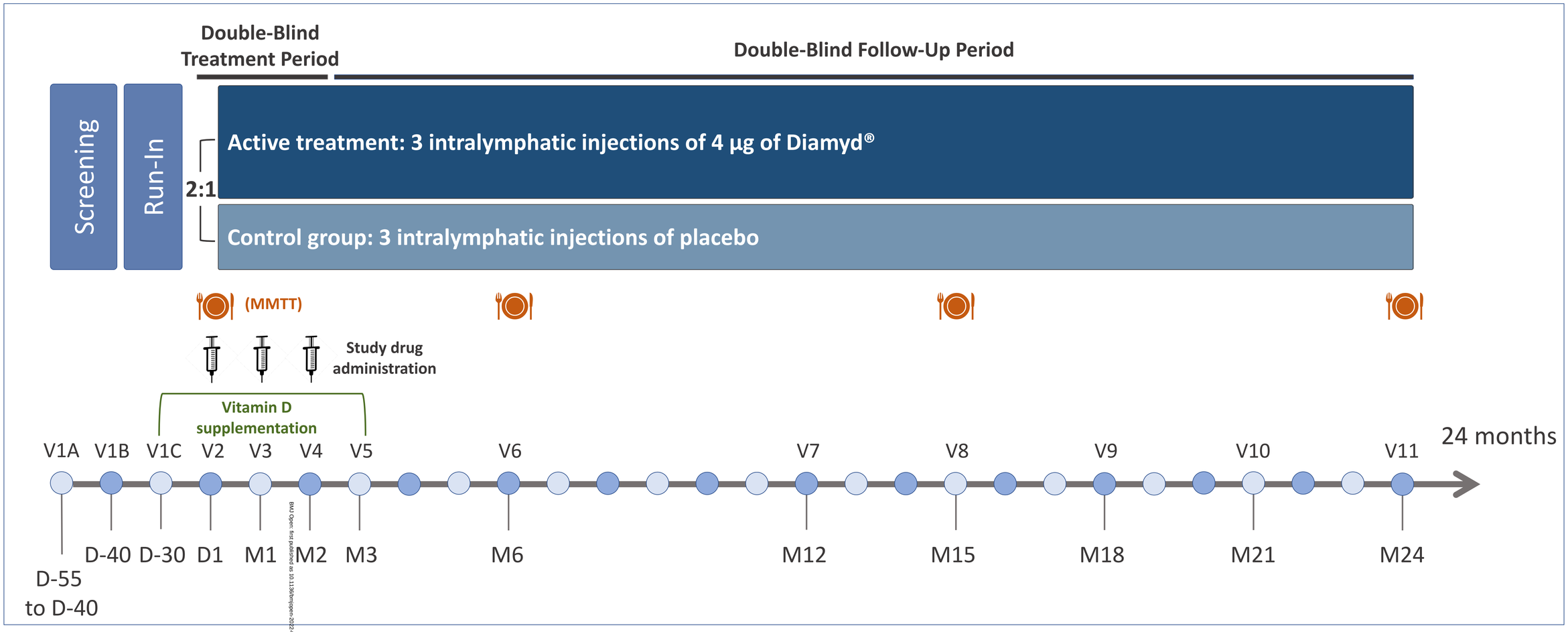
Figure Legend:

Figure 1 - Schematic overview of the study design



Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

A Phase III, Randomized, Double-blind, Placebo-controlled, Multicenter Trial to Evaluate the Efficacy and Safety of rhGAD65 to Preserve Endogenous Beta Cell Function in Adolescents and Adults with Recently Diagnosed Type 1 Diabetes, Carrying the Genetic HLA DR3-DQ2 Haplotype – The DIAGNODE-3 study protocol.

Johnny Ludvigsson[1,2], Linnea Eriksson[3], Christoph Nowak[3,4], Pedro F. Teixeira[3], Martina Widman[3], Anton Lindqvist[3], Rosaura Casas[1], Marcus Lind[5,6,7], Ulf Hannelius[3]

DIAGNODE-3 Statistical Analyses Plan

Populations for analyses

The following analysis sets will be used for the statistical analysis and presentation of data:

- The screened set will consist of all patients who were screened for participation in this study. The screened set will be used for presentation of study disposition of patients.
- The randomized set will consist of all patients who were randomized.
- The safety set (SAF) will consist of all randomized patients who received at least one injection. Patients will be analyzed according to treatment received rather than randomized. If a patient received more than one randomized treatment, they will be analyzed and included in summaries according to the treatment they received the most. Patients receiving no study treatment will be excluded, as will patients who have no post-dose safety assessments. Safety analyses will be based on the SAF.

- The FAS will consist of all randomized patients who have received at least one dose of study medication, a baseline measurement and have at least one post-baseline assessment for any efficacy endpoint. The FAS is the primary analysis dataset, and will be used for all primary, secondary and exploratory efficacy endpoints. Patients in the FAS will contribute to the analysis "as randomized".
- The per protocol set (PPS) will consist of all patients in the FAS who meet the following criteria:
  - Have no important protocol deviations;
  - Completed the treatment phase (Month 24) for the primary end point (i.e., did not discontinue from the trial early);
  - Received all injections of study drug.

C-peptide

The null hypothesis (H_0) is that there is no difference versus the alternative hypothesis (H_1) that there is a difference in the geometric mean ratio (GMR) between the Diamyd-treated group and the placebo-treated group. The null and alternative hypotheses testing can be formalized as follows:

$$H_0: \text{GMR (Diamyd/placebo)} = 1 \quad \text{vs.} \quad H_1: \text{GMR (Diamyd/placebo)} \neq 1$$

where GMR (Diamyd/placebo) is the back-transformed least square mean (LSM) ratio in the relative change from baseline in $AUC_{\text{mean 0-120 min}}$.

Change from baseline will be analyzed using a Restricted Maximum Likelihood-based repeated measures approach (MMRM). The model for analysis will include fixed, categorical effects of treatment, stratification variables, visit, treatment-by-visit interaction, as well as the continuous, fixed covariate of log-transformed baseline C-peptide $AUC_{\text{mean 0-120 min}}$ during an MMTT and the interaction between baseline C-peptide-by-visit, and the fixed continuous covariate of baseline age. Patient identification number will be included as a categorical random effect. An unstructured covariance matrix will be assumed. If this analysis fails to converge, compound symmetry will be tested. The (co)variance structure converging to the best fit, as determined by Akaike's information criterion will be used as the primary analysis. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom.

LSM estimates and 95% CIs will be back-transformed from the natural log scale to the original scale and presented together with nominal p-values. Back-transformed estimates of the treatment difference will provide an estimate of the (Diamyd/placebo)-ratio in the relative change from baseline in $AUC_{\text{mean 0-120 min}}$. A ratio of e.g., 0.8 will mean that the change from baseline to Month 24 in C-peptide level was 20% smaller for Diamyd than for placebo at Month 24.

The primary efficacy analyses will be repeated using the PPS.

HbA1c

If the null hypothesis for the first primary endpoint C-peptide is rejected, then the second primary endpoint HbA1c will be tested. The null hypothesis (H_0) is that there is no difference versus the alternative hypothesis (H_1) that there is a difference in the mean change from baseline to EoS in HbA1c between the Diamyd-treated group and the placebo-treated group. The null and alternative hypotheses testing can be formalized as follows:

$$H_0: \mu\text{Diamyd} = \mu\text{Placebo} \quad \text{vs.} \quad H_1: \mu\text{Diamyd} \neq \mu\text{Placebo}$$

where μ is mean change from baseline to EoS in HbA1c.

If the null hypothesis for the first primary endpoint is not rejected then the hierarchical testing in the overall DR3-DQ2-positive population stops; p-values for the second primary endpoint will be regarded as exploratory.

Change from baseline will be analyzed with the MMRM model and subject to the sensitivity analyses described in Section 0.

HLA DR4-DQ8-negative Subgroup

If either of the co-primary endpoints is not statistically significant in the overall population at a two-sided significance level of 0.04, the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.01 level of significance in an analogous manner to the primary analysis in the overall population. If both co-primary endpoints in the overall population are statistically significant at the two-sided 0.04 level, then the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.05 level of significance in an analogous manner to the primary analysis in the overall population.

Statistical Analyses of Other Endpoints

Analyses of Secondary Endpoints

The following secondary efficacy endpoint will be analyzed with a similar MMRM model as the primary efficacy endpoint (details on log-transformations will be provided in the SAP):

- Change in time in glycemic target range 3.9 to 10 mmol/L (70 to 180 mg/dL) [evaluated from CGM data] between baseline and 24 months.

A specific section of the SAP will lay out in detail the processing and statistical analysis of the raw CGM device data.

The following secondary efficacy endpoint will be analyzed using the Cochran/Mantel-Haenszel Test stratified by the stratification variables; 95% CIs will be calculated according to the Clopper-Pearson method:

- Proportion of patients with IDAA1c ≤9 (partial remission) at 24 months.

The following secondary efficacy endpoints will be assessed using Poisson regression, including stratification variables; rate ratios with 95% CI and p-value will be given:

- Number of episodes per patient of severe hypoglycemia between baseline and 24 months.
- Number of episodes per patient of DKA between baseline and 24 months.

Analyses of Exploratory Endpoints

The following exploratory endpoint variables will be analyzed with a similar MMRM model as the primary efficacy endpoint (details on log-transformations will be provided in the SAP):

- Change from baseline to Month 24 in IDAA1c.
- Change from baseline to Month 24 in exogenous insulin requirements based on total number of units of insulin per kilogram body weight per day.
- Change in time in severe hypoglycemic range <3.0 mmol/L (50 mg/dL) [evaluated from CGM data] between baseline and Month 24.
- Change in time in hypoglycemic range 3.0 to 3.8 mmol/L (50 to 69 mg/dL) [evaluated from CGM data] between baseline and Month 24.
- Change in glycemic variability as measured by %CV [evaluated from CGM data] between baseline and Month 24.
- Change in (fasting, maximal, and stimulated) C-peptide measured at 0, 30, 60, 90, and 120 minutes during MMTT at Month 24.
- Change in serum GAD65A titers between baseline and Month 24.
- Change in QoL evaluated by PRO measures (PedsQL), family impact, generic and diabetes module with parent proxy between baseline and Month 24.
- Change from baseline to Month 24 in BMI.

The following exploratory endpoint will be assessed using Poisson regression, including stratification variables; rate ratios with 95% CI and p-value will be given:

- Number of episodes per patient of mild/moderate hypoglycemia between baseline and Month 24.

The following exploratory endpoints will be analyzed using the Cochran/Mantel-Haenszel Test stratified by the stratification variables; 95% CIs will be calculated according to the Clopper-Pearson method:

- Proportion of patients with a stimulated 90 min C-peptide level above 0.2 nmol/L (0.6 ng/mL) at Month 24.
- Proportion of patients with new onset hyperthyroidism, hypothyroidism, and celiac disease.
- Proportion of patients with increase or decrease in medication usage for treatment of hyperthyroidism and hypothyroidism in those with such disorders at baseline.
- Proportion of patients who change insulin delivery method during the study (MDI/CSII/semi/closed loop system).

Analysis of Safety and Immunological Endpoints

The safety endpoints will be evaluated based on the SAF

Immunological endpoints will be summarized descriptively, including p-values from non-parametric statistical tests (details to be provided in the SAP).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	18
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 & 18
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10

Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5-7
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	1, 9-10
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	Table 1 and 2

		individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10, figure 1
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	14
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	13-14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9-10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8, 15
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended	Figure 1, Table 3

		(see Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9, 15
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to	10

		interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13-14
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16
Data management	#19	Plans for data entry, coding, security, and storage,	N/A

		including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15-16
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16, appendix
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15-16, Appendix
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A

Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Abstract, 18
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9, Table 3
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in	N/A

		order to protect confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

A Phase III, Randomized, Double-blind, Placebo-controlled, Multicenter Trial to Evaluate the Efficacy and Safety of rhGAD65 to Preserve Endogenous Beta Cell Function in Adolescents and Adults with Recently Diagnosed Type 1 Diabetes, Carrying the Genetic HLA DR3-DQ2 Haplotype – The DIAGNODE-3 study protocol

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A Phase III, Randomized, Double-blind, Placebo-controlled, Multicenter Trial to Evaluate the Efficacy and Safety of rhGAD65 to Preserve Endogenous Beta Cell Function in Adolescents and Adults with Recently Diagnosed Type 1 Diabetes, Carrying the Genetic HLA DR3-DQ2 Haplotype – The DIAGNODE-3 study protocol.

Johnny Ludvigsson[1,2], Linnea Eriksson[3], Christoph Nowak[3,4], Pedro F. Teixeira[3], Martina Widman[3], Anton Lindqvist[3], Rosaura Casas[1], Marcus Lind[5,6,7], Ulf Hannelius[3]

Affiliations:

a) Division of Pediatrics, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, Linköping University, Linköping, Sweden
b) Crown Princess Victoria Children´s Hospital, Linköping, Sweden
c) Diamyd Medical AB, Stockholm, Sweden
d) Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Huddinge, Sweden
e) Department of Molecular and Clinical Medicine, University of Gothenburg, Sweden
f) Department of Internal Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
g) Department of Medicine, NU-Hospital Group, Trollhättan and Uddevalla, Sweden

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Corresponding author:

Professor Johnny Ludvigsson

Division of Pediatrics, Department of Biomedical and Clinical Sciences, Faculty of Medicine and Health Sciences, and Crown Princess Victoria Children's Hospital, Linköping University, SE-58185 Linköping, Sweden

Email: Johnny.Ludvigsson@liu.se

ABSTRACT

Introduction

Type 1 diabetes (T1D) is an autoimmune disease leading to the destruction of the insulin-producing beta cells resulting in insulin deficiency and hyperglycemia. Today, no approved therapy exists to halt this detrimental immunologic process. In a recent Phase 2b study, intralymphatic administration of recombinant human glutamic acid decarboxylase (rhGAD65) adsorbed to Alhydrogel adjuvant to individuals recently diagnosed with T1D and carrying the HLA DR3-DQ2 haplotype showed promising results in preserving endogenous insulin secretion, confirming the results of a large meta-analysis of three randomized placebo-controlled trials of subcutaneous rhGAD65. The aim of the current precision medicine Phase 3 study is to determine whether intralymphatic administration of rhGAD65 preserves insulin secretion and improves glycaemic control in presumed responder individuals with recently diagnosed T1D carrying HLA DR3-DQ2.

Methods and analysis

Individuals ≥12 and <29 years old recently diagnosed with T1D (<6 months) will be screened for the HLA DR3-DQ2 haplotype, endogenous insulin production estimated by fasting C-peptide and presence of GAD65 antibodies. 330 patients are planned to be randomized to three monthly intralymphatic injections of rhGAD65 or placebo (both accompanied by oral Vitamin D supplementation), followed by 22 months of follow-up. The study is powered to detect a treatment effect in the two co-primary endpoints; change from baseline in $AUC_{(0\text{-}120min)}$ C-peptide levels during a mixed meal tolerance test, and change from baseline in glycemic control estimated by HbA1c at 24 months. Secondary endpoints include effects on glucose patterns collected by masked continuous glucose monitoring, proportion of patients in partial remission, and number of episodes of severe hypoglycemia and/or diabetic ketoacidosis.

Ethics and dissemination

The trial is approved by Ethics Committees in Poland (124/2021), Netherlands (R21.089), Sweden (2021-05063), Czech Republic (EK-1144/21), Germany (2021361), and Spain (21/2021). Results will be published in international peer-reviewed scientific journals and presented at national and international conferences.

Trial registration:

EudraCT identifier: 2021-002731-32

NCT identifier: NCT05018585

Keywords: Type 1 diabetes, immune intervention, rhGAD65, intralymphatic, HLA DR3-DQ2, Phase 3, antigen specific therapy, precision medicine

Article Summary

Strengths and limitations of this study

- The current study is a large, international, multi-centre, randomized double-blind placebo-controlled trial.
- The study is adequately powered to detect a treatment effect on two clinically important co-primary endpoints; preservation of beta cell function and glycaemic control (HbA1c).
- The total study duration of 24 months should allay concerns about confounding from the so-called honeymoon period in recently diagnosed T1D.
- This is the first Phase 3 trial in T1D using a precision medicine approach, limiting recruitment to the identified HLA DR3-DQ2 responder population to rhGAD65 treatment.
- To fully understand the magnitude of a possible beneficial treatment effect, additional follow-up over several years might be needed to see the benefits of even minimum residual beta cell function.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disorder in which the immune system attacks the insulin producing beta cells in the pancreas. By the time an individual is diagnosed with T1D, 70-90% of beta cell function has generally been lost. The destruction of the pancreatic beta cells in T1D is associated with cellular immune responses towards the pancreatic islet cells (1). Autoantibodies directed against glutamic acid decarboxylase (GAD) with a molecular mass 65 kDa (GAD65A), insulinoma-associated protein 2 (IA-2A), insulin (IAA), or zinc transporter antigen T8 (ZnT8A) precede the clinical onset of the disease (1).

T1D treatment consists of lifelong administration of exogenous insulin, which does not satisfactorily prevent neither acute nor long-term complications. The disease has a devastating impact on the quality of life (QoL) of the affected person and their family due to the constant stress of adjusting blood sugar and the common acute and life-threatening consequences of imperfect control - diabetic ketoacidosis (DKA) and severe hypoglycaemia (2-4). In addition, many individuals with T1D experience over the long term both macro- and microvascular complications affecting the heart, nerves, eyes and kidneys, putting them at risk of blindness, kidney failure and myocardial ischemia (5, 6). A recent article (7) concluded that patients who received their diagnosis before the age of 10 years had a shortened lifespan by 14 years for males and 18 years for females. Early-onset T1D was also found to be associated with a 30 times increased risk of serious cardiovascular outcomes and for women, this risk was 90 times higher compared to non-diabetic control persons (7). Even with good long-term blood glucose control (HbA1c ≤52 mmol/mol), the risk of premature death for any T1D patient is found to be twice as high as for healthy individuals and up to eight times higher for patients with poor glycemic control (8).

There is currently no approved treatment preventing the destruction of beta cells. Insulin replacement therapy is the standard-of-care treatment, and despite the development of new insulins, new technologies for insulin administration and blood glucose diagnostics, patient targets for long-term blood glucose are currently met less frequently than 5 years ago in some populations in the US (9). Any intervention which can stop or delay the loss of beta cell function would likely provide protection against hypoglycemia and ketoacidosis, improve metabolic control, decrease blood glucose fluctuations, facilitate treatment and delay and/or reduce micro and macrovascular complications of diabetes (10-13). Additionally, decreasing the autoimmune destruction of beta cells could allow for beta cell replenishment, either through regeneration or transplantation.

The most efficient immune therapy for preservation of beta cell function is so far treatment with anti-CD3 monoclonal antibodies (teplizumab) (14, 15). TNF-alfa inhibitors (16), anti-thymocyte globulin (ATG) (17), alefacept (18) and rituximab (19) have also demonstrated some efficacy in preserving beta cell function, but often these therapies have adverse events, serious risks and impose a heavy treatment burden, including e.g. several days of intravenous infusions. An alternative approach is treatment with autoantigen immunotherapies, even though most clinical trials with autoantigen immunotherapies have failed to meet their primary endpoints or shown inconclusive results (20-23).

Over the last two decades, however, an important development in the field has meant a shift away from a one-size-fits-all approach to T1D pathophysiology towards a more individualized, precision medicine approach that recognizes inter-individual heterogeneity in T1D (24). The concept of disease heterogeneity has recently been extended to the concept of endotypes; that is, subtypes of T1D with distinct underlying pathobiological mechanisms, which should be considered in the design of clinical trials (25). For instance, the appearance of GAD65 autoantibodies (GADA) as the first autoantibody is linked to the Human Leukocyte Antigen (HLA) DR3-DQ2 haplotype, while emergence of insulin autoantibodies as the first antibody is linked to HLA DR4-DQ8 (25, 26). As a consequence, applying the same intervention targeting a specific pathophysiologic mechanism across an entire population ignores the fact that subgroups of patients whose disease is driven by the targeted mechanism may respond particularly well, whilst others show no response, resulting in apparently absent treatment effects across the entire population.

GAD antigen-specific immunotherapy to preserve endogenous insulin secretion

GAD65 is a major autoantigen in autoimmune diabetes and clinical administration of purified recombinant human GAD65 rhGAD65 aims to intervene in the autoimmune process in T1D. The rhGAD65 is adsorbed to Alhydrogel® (aluminium hydroxide particles) and formulated in phosphate buffer with mannitol. The intended mode of action is to slow or prevent autoimmune destruction of pancreatic beta cells by modulation of immune responses to GAD65. Inconsistent results observed in trials testing subcutaneous administration of rhGAD65 spurred the evaluation of alternative approach to improve treatment efficacy (20-22, 27). DIAGNODE-1 (28, 29), a Phase 1/2a open-label pilot combination trial evaluated an alternative administration route, with three doses of 4 µg rhGAD65 administered directly into inguinal lymph nodes, in combination with oral Vitamin D in 12 patients (12 to 30 years of age) recently diagnosed with T1D. All patients were followed for 30 months. The positive results of the DIAGNODE-1 trial

(29) supported further development in a Phase 2b trial (DIAGNODE-2), a randomized and placebo-controlled trial testing the intralymphatic administration in 109 patients (12 to 24 years of age) recently diagnosed with T1D. Importantly, based on the concept of heterogeneity of disease mechanism, a meta-analysis of three previous RCTs testing subcutaneous rhGAD65 was performed. The analysis showed that clinical efficacy is mainly seen in patients with HLA DR3-DQ2, and an even more pronounced treatment effect was seen in those individuals with HLA DR3DQ2 without HLA DR4-DQ8 though no clinical efficacy was observed in the full population(30). Due to the identification of HLA DR3-DQ2 patients as the responder population, the statistical analysis plan of the then on-going DIAGNODE-2 study was amended before database lock to include analyses of the primary and secondary endpoints in the HLA DR3-DQ2 subgroup in the topline results. At 15 months of follow up in DIAGNODE-2, rhGAD65 treatment showed a significant positive treatment effect in the pre-specified genetic subgroup of patients positive for HLA DR3-DQ2 of 55.7% (p-value 0.0078), i.e. that on average, the primary end point stimulated C-peptide secretion $AUC_{mean\ 0\text{-}120\ min}$ declined by 55.7% less in patients treated with rhGAD65 compared to patients treated with placebo. For patients positive for HLA DR3-DQ2, C-peptide $AUC_{mean\ 0\text{-}120\ min}$ declined approximately 28% over 15 months in the rhGAD65 group compared to about 58% for placebo (31). There were corresponding trends, though not statistically significant, in improvement in the secondary efficacy variables HbA1c, IDAA1c and exogenous insulin use after 15 months in the HLA DR3-DQ2-positive patients treated with rhGAD65 when compared to placebo.

Results from an updated meta-analysis (manuscript in preparation) which added data from DIAGNODE-2 to the previous meta-analysis (30) showed that in patients carrying the HLA DR3-DQ2-haplotype, a statistically significant treatment effect on change in AUC C-peptide of 48.3% for the subjects receiving 3 or 4 injections of rhGAD65 ($p < 0.0001$) and 36.1 % for the 2-4 injections ($p = 0.0316$) . In addition to this, a statistically significant treatment effect on change in HbA1c of -4.789 mmol/mol for the subjects receiving 3 or 4 injections of rhGAD65 ($p = 0.0044$) and -3.120 mol/mol for subjects receiving 2-4 injections ($p = 0.032$). The data also reconfirmed previous findings that an even more pronounced treatment effect (on both change in AUC C-peptide and HbA1c) was seen in those individuals with HLA DR3DQ2 without HLA DR4-DQ8. Intralymphatic rhGAD65 injections were well tolerated and considered safe, consistent with prior clinical trial findings (31).

Objectives

The primary objective is to evaluate the effect of three doses of rhGAD65 compared to placebo in terms of (1) beta cell function; and (2) glycemic control in adolescents and adults recently diagnosed with T1D, who carry the HLA DR3-DQ2 haplotype. Secondary objectives are to compare the effect of rhGAD65 to placebo treatment with respect to the effects on other diabetes disease management indicators and long-term safety.

METHOD AND ANALYSIS

Overall Study Design

DIAGNODE-3 is a Phase III randomized, double-blind, placebo-controlled, international, multicenter, parallel-arm, 24-month trial in adolescents and adults with recently diagnosed T1D, carrying the HLA DR3-DQ2 haplotype. Overall study design is shown in Figure 1. The study is registered on Clinicaltrials.gov (NCT05018585). The study is expected to take 4.5 years to complete. This includes an intervention with follow-up for 24 months.

Throughout the study duration, all patients will receive standard-of-care routine treatment for their diabetes according to ADA guidelines (amended as appropriate to reflect local standard of care).

Screening and Run-In Period

Patients deemed eligible and/or their parent(s)/legal guardian(s) will have the study explained to them and will receive written patient information. After having had the time to review the study, they will have the opportunity to ask questions to the investigational team. After this, if the patient agrees to participate, they will sign and date the written informed consent form and for patients who are minors, both age-appropriate assent (according to local regulations) and parent's/caregiver's consent is collected. Patients and their parent(s)/legal guardian(s), when applicable, will provide written informed consent before any study-related procedures are performed. The patient and/or their parent(s)/legal guardian(s) will then receive a copy of the signed and dated patient information. Detailed study assessments are shown in Table 1. HLA genotyping of the patient is performed at the first Screening visit after preliminary eligibility is confirmed. If the patient is carrying the HLA DR3-DQ2 haplotype, the patient will attend the second Screening visit (Visit 1B) to perform the remaining Screening procedures. After Screening, patients deemed eligible will proceed to the Run-In period (beginning at Visit 1C) undergo masked CGM for 14 days, receive diabetes education and collect self-reported diabetes information in their eDiary.

Patients with a Screening Vitamin D level <100 nmol/L (40 ng/mL) will start oral Vitamin D supplementation (2000 IU daily) beginning at Visit 1C, 30 days prior to randomization. During the period of supplementation, Vitamin D should be discontinued temporarily if the level exceeds 125 nmol/L (50 ng/mL) and may be resumed when levels are <100 nmol/L (40 ng/mL).

TABLE 1 SCHEDULE OF ASSESSMENTS IN DIAGNODE-3

	Screening		Run-In	Double-Blind Treatment Period			Double-Blind Follow-Up Period								
VISIT	**1A**	**1B**	**1C**	**2 (Baseline)**	**3**	**4**	**5** ☎	**6**	**7**	**8**	**9**	**10** ☎	**11 EoS**	**ET**	**UNS Visit**
Trial Day	**-55 to -40**	**-40**	**-30**	**0**	**30**	**60**	**90**	**180**	**360**	**450**	**540**	**630**	**720**		
Trial Month	**-2**		**-1**	**1**	**1**	**2**	**3**	**6**	**12**	**15**	**18**	**21**	**24**		
Visit Window (days)		**±5**	**±5**	**0**	**±5[l]**	**±5[l]**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**		
Informed consent	X														
Informed consent for genomic sub-study									X						
Review eligibility criteria	X	X	X	X											
Demographic information	X														
Medical history	X	X													
Family history of T1D	X														
Examinations															
Physical examination		X complete		X limited	X limited	X limited		X limited	X limited	X complete	X limited		X complete	X complete	X limited
Neurological assessment		X		X	X	X		X	X	X	X		X	X	X
Tanner staging (if <18 years)				X				X	X		X		X		
Vital signs		X	X	X	X	X		X	X	X	X		X	X	X
Height and weight		X		X	X	X		X	X	X	X		X	X	X
Trial Activities															
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Diabetes education			X			X			X		X				
Adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X
Vitamin D supplementation if <100 nmol/L (40 ng/mL)			X Start	X	X	X	X End								
MMTT[j]				X				X		X			X		
Continuous glucose monitoring (blinded)			X					X		X			X		
Randomization				X											
Intralymphatic study drug administration				X	X	X									
Injection site inspection, site staff				X	X	X									
Monitoring of target and adjacent lymph nodes using ultrasound				X	X	X									
eDiary															
Distribution of eDiary			X												
Review of eDiary				X	X	X	X	X	X	X	X	X	X	X	X
Glucose events collection in eDiary			X	X	X	X	X	X	X	X	X	X	X		X

	Screening		Run-In	Double-Blind Treatment Period			Double-Blind Follow-Up Period								
VISIT	**1A**	**1B**	**1C**	**2 (Baseline)**	**3**	**4**	**5** ☎	**6**	**7**	**8**	**9**	**10** ☎	**11 EoS**	**ET**	**UNS Visit**
Trial Day	**-55 to -40**	**-40**	**-30**	**0**	**30**	**60**	**90**	**180**	**360**	**450**	**540**	**630**	**720**		
Trial Month	**-2**		**-1**	**1**	**1**	**2**	**3**	**6**	**12**	**15**	**18**	**21**	**24**		
Visit Window (days)		**±5**	**±5**	**0**	**±5[l]**	**±5[l]**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**		
Review of injection site inspection, eDiary					X	X	X								
Patient-reported activities in eDiary			X					X		X			X		
Daily insulin collection in eDiary			X	X	X	X	X	X	X	X	X	X	X		
Blood Samples for Diabetes Status															
Fasting C-peptide		X		X	X	X		X	X	X	X		X	X	
MMTT-induced C-peptide				X				X		X			X		
MMTT-induced glucose				X				X		X			X		
HbA1c		X		X	X	X		X	X	X	X		X	X	
Fasting plasma glucose		X		X	X	X		X	X	X	X		X	X	
Blood and Urine Sampling for Safety, Genetics, Vitamin D Levels and Immunology															
Hematology		X		X				X	X	X	X		X	X	
Clinical chemistry		X		X	X	X		X	X	X	X		X	X	
Lipid panel (fasting)				X						X			X		
TSH (reflex Free T4), TPO		X								X			X		
Transglutaminase antibody titer				X						X			X		
IA-2 antibody titer				X						X			X		
Vitamin D[s]		X		X	X	X		X	X	X	X		X		
Pregnancy testing, (FOCBP only)		X serum		X urine	X urine	X urine		X urine	X urine	X urine	X urine		X urine		
FSH, LH		X		X		X				X	X		X		
Microalbuminuria (UACR)				X				X	X	X	X		X		
GAD65A titer		X		X	X	X		X	X	X	X		X	X	
HLA class II genotyping	X														
COVID-19 antibody testing				X						X			X		
Other immunologic parameters				X	X	X		X		X			X		
DNA sample collection for genomic sub-study									X						
Quality of Life Assessments															
PedsQL questionnaires				X				X		X			X		

Abbreviations: AE = adverse event; BP = blood pressure; CGM = continuous glucose monitoring; COVID-19 = Coronavirus Disease 2019; DKA = diabetic ketoacidosis; DNA = deoxyribonucleic acid; eCRF = electronic case report form; eDiary = electronic diary; EoS = end of study; ET = early termination; FOCBP = females of childbearing potential; FSH = follicle stimulating hormone; GAD65A = glutamic acid decarboxylase with molecular mass 65 kDa antibody; HbA1c = hemoglobin A1c; HCG = human chorionic gonadotropin; HLA = human leukocyte antigen; HR = heart rate; IA-2 = insulinoma-associated protein 2; ICF = informed consent form; LH = luteinizing hormone; MMTT = mixed meal tolerance test; T1D = type 1 diabetes; T4 = thyroxine; TPO = thyroid peroxidase antibody; TSH = thyroid stimulating hormone; UACR = urine albumin to creatinine ratio; UNS = unscheduled.

Double-Blind Treatment Period and Long Term Follow up.

At Visit 2, patients will be randomized 2:1 to one of the following two treatment groups:

Treatment Group 1:	3 intralymphatic injections of 4 µg (0.1 mL) of rhGAD65 administered on Days 0, 30 and 60
Treatment Group 2:	3 intralymphatic injections of 0.1 mL placebo administered on Days 0, 30 and 60

Randomization will be performed by an Interactive Web Response Systems and stratified by HLA subgroup (presence or absence of HLA DR4-DQ8) and by region. The maximum number of adults (>18 years old) recruited into the trial is 160. rhGAD65 or placebo injections will be administered in the inguinal lymph node by qualified personnel with the help of ultrasound. Vitamin D levels will be monitored throughout the trial. Vitamin D oral supplementation (2000 IU daily) will be administered from Day -30 (Visit 1C) through Day 90 for a total of 120 days for patients with a level <100 nmol/L (40 ng/mL) at Screening. All patients will continue to receive intensive insulin therapy via multiple daily injections or via CSII. Safety will be assessed via physical examinations, neurological assessments, vital signs, clinical laboratory assessments, injection site reactions, and AEs. After the double blinded treatment period of 2 months, patients will be followed in a blinded manner for 22 months. An independent DSMB will be appointed to review unblinded safety data (at least twice a year).

Study Population

Individuals between ≥12 and <29 years old, will be eligible for enrollment if they have been diagnosed with T1D within the previous 6 months at the time of screening, positive for the HLA DR3-DQ2 haplotype, fasting C-peptide is ≥0.12 nmol/L (0.36 ng/mL) and seropositive for GADA. Full list of inclusion and exclusion criteria is shown in Table 2 and Table 3.

TABLE 2 - INCLUSION CRITERIA IN THE DIAGNODE-3 STUDY.

Patients are eligible to be included in this study if all the following criteria apply:	
1.	Must be capable of providing written, signed, and dated informed consent; and for patients who are minors, age-appropriate assent (performed according to local regulations) and parent/caregiver consent.
2.	Males and females aged ≥12 and <29 years old at the time of Screening.
3	Diagnosed with T1D (according to the American Diabetes Association [ADA] classification) ≤6 months at the time of Screening.
4	Possess the HLA DR3-DQ2 haplotype (all patients will be tested; prior genetic testing results will not be accepted).
5	Fasting C-peptide ≥0.12 nmol/L (≥0.36 ng/mL) on at least one occasion (maximum two tests on different days during the Screening period).
6	Possess detectable circulating GAD65 antibodies (lowest level of detection defined by the method used by the central laboratory).
7	Possess HbA1c levels between 35 to 80 mmol/mol (5.4 to 9.5%) on at least one occasion prior to randomization (maximum one additional test within one month from Visit 1B).
8	Be on a stable insulin dose or insulin dosing regimen for one month prior to inclusion with limited fluctuation of daily insulin requirement based on investigator's assessment. For example, if the average insulin dose/kg/24h over a 7-day period compared to the previous 7-day period does not vary more than approximately 15% and/or if the daily insulin dose does not vary more than 0.1 U/kg/24h, the dose can be considered stable. Individuals that are diagnosed with T1D according to the ADA classification but are not taking insulin are eligible to participate.
9	i) Females of childbearing potential (FOCBP) must agree to avoid pregnancy and have a negative pregnancy test performed at the required study visits. FOCBP must agree to use highly effective contraception, during treatment and, until 90 days after the last administration of study medication. ii) Male patients must agree to remain abstinent from heterosexual sex during treatment and for 90 days after treatment or, if sexually active, to use two effective methods of birth control (e.g., male uses a condom and female uses contraception) during and for 90 days after treatment.

TABLE 3 - EXCLUSION CRITERIA IN THE DIAGNODE-3 STUDY

Patients are not eligible to be included in this study if any of the following criteria apply:	
1	Participation in any other trial aimed to influence beta cell function from time of diagnosis of T1D.
2.	Treatment with any oral or non-insulin injectable anti-diabetic medication within 3 months prior to Screening.
3.	History of maturity-onset diabetes of the young (MODY).
4.	Pancreatic surgery, chronic pancreatitis, or other pancreatic disorders that could result in decreased beta cell capacity (e.g., pancreatogenous diabetes).
5.	History of DKA or severe hypoglycemia requiring hospitalization within one month before Screening, or severe episodes of hypoglycemia requiring third party assistance within one month before Screening.
6.	Signs or symptoms suggesting very poorly controlled diabetes e.g., ongoing weight loss, polyuria or polydipsia.
7.	Hematologic condition that would make HbA1c uninterpretable including: a) Hemoglobinopathy, with the exception of sickle cell trait or thalassemia minor; or chronic or recurrent hemolysis. b) Donation of blood or blood products to a blood bank, blood transfusion or participation in a clinical study requiring withdrawal of >400 mL of blood during the 8 weeks prior to the Screening visit. c) Significant iron deficiency anemia. d) Heart malformations or vaso-occlusive crisis (VOC) leading to increased turnover of erythrocytes.
8.	Treatment with marketed or over-the-counter Vitamin D at the time of Screening and unwilling to abstain from such medication during the 120 days when the patient will be supplemented with the study-provided Vitamin D. A patient currently taking Vitamin D at the time of Screening must be willing to switch to the study-provided Vitamin D treatment and to administer it per the study requirements.
9.	Any clinically significant history of an acute reaction to a vaccine or its constituents (e.g., Alhydrogel).
10.	Treatment with any (live or inactive) vaccine, including influenza vaccine and Coronavirus Disease 2019 (COVID-19) vaccine, within 4 weeks prior to planned first study dose of study drug; or planned treatment with any vaccine up to 4 weeks after the last injection with study drug.
11.	Any acute or chronic skin infection or condition that would preclude intralymphatic injection.
12.	Recent (past 12 months) or current treatment with immunosuppressant therapy, including chronic use of glucocorticoid therapy. Inhaled, topical, and intranasal steroid use is acceptable. Short courses (e.g., ≤5 days) of oral or intra-articular injections of steroids will be permitted on trial.
13.	Continuous/chronic treatment with prescribed or over-the-counter anti-inflammatory therapies. Short-term use (e.g., <7 days) is permissible, for example to treat a headache or in connection with a fever.
14.	Known or suspected acute infection, including COVID-19 or influenza, at the time of Screening or within 2 weeks prior to Screening. After confirmed recent COVID-19 infection, a negative polymerase chain reaction test will be required before randomization.
15.	A history of epilepsy, head trauma or cerebrovascular accident, or clinical features of continuous motor unit activity in proximal muscles.
16.	Known diagnosis of human immunodeficiency virus (HIV), hepatitis B or hepatitis C infection. Patients with previous hepatitis C infection that is now cured may be eligible.
17.	Any clinically significant concomitant medical condition
18.	History of significant hepatic disease
19.	Estimated glomerular filtration rate (eGFR) calculated by Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) for those >18 years old, and by the Schwartz equation for those 12 to 18 years old, <90 mL/min per 1.73 m or rapidly progressing renal disease.
20.	Patients with hypothyroidism or hyperthyroidism must be on stable treatment for at least 3 months prior to Screening (with normal free thyroxine [T4] levels if hypothyroid).
21.	Any clinically significant abnormal findings during Screening, and any other medical condition(s) or laboratory findings that, in the opinion of the investigator, might jeopardize the patient's safety or ability to complete the trial.
22.	History of malignancy not in remission within the last 5 years other than adequately treated basal cell or squamous cell skin cancer or cervical carcinoma in situ.
23.	Patients with any mental condition rendering him/her unable to understand the nature, scope and possible consequences of the trial, and/or evidence of poor compliance with medical instructions at Screening or showing non-compliance during the Run-In Period.
24.	A history of alcohol or drug abuse or dependence within the past 12 months based on DSM IV criteria.
25.	Current or previous participation in a trial of Diamyd.
26.	Participation in a clinical trial involving administration of an investigational drug in the past 3 months or 5 half-lives (whichever is longer) prior to first dosing of study drug or during the trial.
27.	Females who are breastfeeding, pregnant or plan to become pregnant during the trial.
28.	Patients who in the opinion of the investigator will not be able to follow instructions and/or follow the study procedures or patients that are unwilling or unable to comply with the provisions of this protocol.
29.	An employee or immediate family member of an employee of Diamyd Medical AB.

Study Assessments

Demographics and study procedures

Demographics, baseline data medical history and family history of T1D will be collected at screening. Medical examinations (i.e physical, neurological, and vital signs) will be performed at all on site visits. Patients will also be provided with an eDiary to collect self-reported data on daily insulin dose, injection site reactions, significant glucose events (mild/moderate/severe hypoglycaemic events and DKAs), as well as mealtimes and physical activity. Patients and caregivers (if applicable) will answer the Pediatric Quality of Life Inventory (PedsQL) questionnaires at four visits between baseline and Month 24 to assess Quality of Life. Timings of all assessment can be found in Table 1.

Clinical Laboratory assessments

Laboratory assessments of diabetes status

The timing of all study assessments is presented in Table 1 All laboratory parameters will be analyzed at a central laboratory. A 2-hour MMTT following an overnight fast (>10 hours) will be performed at baseline and at month 6, month 15 and month 24. Meal stimulated plasma glucose and C-peptide levels will be assessed throughout the MMTT (32).

Patients should come to the study site following an overnight fast (>10 hours) and have a plasma glucose level between 3.5 to 12 mmol/L (63 to 216 mg/dL) at home in the morning. Patients are allowed to take basal-insulin day/night before, but not in the morning before the MMTT. Patients should also not administer any short/direct acting insulin within 6 hours before the MMTT. Patients with CSII (insulin pump) must continue with their basal dose insulin, but not add any bolus dose during the last 6 hours before the MMTT.

Samples for HbA1c will be analyzed at a National Glycohemoglobin Standardization Program (NGSP) certified central laboratory. Results will be reported in both International Federation of Clinical Chemistry (IFCC) (mmol HbA1c/mol Hb) and NGSP (% HbA1c) units. Serum samples for fasting glucose and fasting C-peptide levels will also be collected and analyzed throughout the trial.

Safety and other laboratory assessments

All patients will undergo HLA class II genotyping to assess the presence of HLA haplotypes DR3-DQ2 and DR4-DQ8 during the screening procedure. Samples will also be collected for clinical chemistry, hematology, urinalysis, lipids (total cholesterol, LDL-C, HDL-C, and

triglycerides), Vitamin D, Thyroid stimulating hormone, Thyroid peroxidase antibody, Transglutaminase antibody, IA-2 antibody, GAD65 antibody, SARS-CoV-2 antibody, and for females; human chorionic gonadotropin, follicle stimulating hormone and luteinizing hormone. Timing of the assessments can be found in Table 1 and all samples will be analyzed at a central laboratory.

Immunology Assessments

Timing of immunological assessments is indicated in Table 1. GAD65 antibody titers will be measured at a central laboratory. Additional variables to evaluate the influence of treatment on the immune system include GAD65 antibody isotypes, IA-2 antibodies, and cell mediated immune response by proliferation and cytokine secretion upon GAD65 stimulation of PBMC.

Continuous Glucose Monitoring

CGM will be performed for 14 days during the Run-In Period (following Visit 1C) and at three other timepoints during the trial. The timing of the distribution of the glucose monitoring system and assessments is presented in Table 1. The FreeStyle Libre Pro/Pro iQ devices are intended for use only by healthcare professionals, with the patients being blinded to the CGM sensor readings. The devices will be used for data collection during the clinical trial but not to inform decisions on diabetes management and therapy adjustments. Patients will be allowed to use an unblinded CGM device to manage their diabetes and adjust the therapy based on the glucose levels registered.

Time Period and Frequency for Collecting AE and SAE Information

Any worsening in the patient's condition after administration of study drug and up to the end of study or early termination visit should be considered an AE. All AEs will be collected throughout the whole study period (starting from Visit 1C), reviewed and assessed for causality by the investigators at the time points specified in Table 1. Injection site reactions will be collected during the 7 days following study drug injections (Visits 2, 3 and 4), starting the day after the injection. Injection site reactions persisting after 7 days should be reported as an AE.

Statistical considerations

Sample Size and Power

The primary efficacy analysis will be performed in the Full Analysis Set (FAS). The primary efficacy variables will be (1) change from baseline to Month 24 in log-transformed C-peptide $AUC_{mean\ 0\text{-}120\ min}$ during an MMTT and (2) change from baseline to Month 24 in mean HbA1c. The co-primary endpoints will be tested in both the overall population and in the subgroup of patients who carry the HLA DR3-DQ2 haplotype and simultaneously do not carry the DR4-DQ8 haplotype (hereafter the HLA DR4-DQ8-negative subgroup). The overall two-sided 5% Type I error rate will be controlled using a fallback procedure. A two-sided 4% alpha will be assigned to the primary efficacy analysis in the overall population. The remaining 1% alpha will be assigned to the primary efficacy analysis in the HLA DR4-DQ8-negative subgroup

A total sample size of 288 patients is planned for a 2:1 randomization to the rhGAD65 and placebo arms, respectively. This achieves 90% power to detect a clinically relevant difference of 40% in geometric mean ratio C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at Month 24 between the rhGAD65 arm and placebo arm using a two-sided test at the 4% significance level. This is based upon a t-test employing natural log transformation of C-peptide ($AUC_{mean\ 0\text{-}120\ min}$) during an MMTT at Month 24 and assumed CV of 0.95 based on simulations of EoS (Month 15) results in the placebo group of the Phase 2b study DIAGNODE-2. Allowing for 12% drop out to Month 24, approximately 330 patients will be randomized.

Statistical Analyses

Primary Efficacy Analysis

Change from baseline in the co-primary endpoints will be analyzed using a Restricted Maximum Likelihood-based repeated measures approach (MMRM). The model for analysis will include fixed, categorical effects of such as for treatment, and stratification variables, as well as interactions effects such as between baseline value-by-visit, and the fixed continuous covariates such as baseline age. Patient identification number will be included as a categorical random effect. An unstructured covariance matrix will be assumed. If this analysis fails to converge, compound symmetry will be tested. The (co)variance structure converging to the best fit, as determined by Akaike's information criterion will be used as the primary analysis. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom. Additional methods of sensitivity analyses may be performed in the event that the proportion of missing values is greater than 5%. These analyses will compare the results from the MMRM model, which assumes that values are missing at random (MAR), with analyses assuming the values are missing not at random (MNAR) such as: return-to-baseline using multiple imputation and tipping point analysis. All sensitivity analyses will be regarded as exploratory, thus no formal adjustment for multiplicity will be performed.

Negligible measurement error is expected, which is assumed to affect all patients, time points and treatment groups equally. The total variance used in the sample size calculation is constructed from the within-subject and the between-subject component of variation. Based on the assumption that the measurement error is the same for everyone, it is therefore accounted for in the total variance estimate.

The co-primary endpoints will be tested sequentially meaning that C-peptide is tested first, and, if significant, HbA1c is tested. Both co-primary endpoints need to meet the statistical significance criterion. The fallback procedure described by Wiens and Dmitrienko (33) will be used to test the primary endpoints in the overall population and in the HLA DR4-DQ8-negative subgroup.

If either of the co-primary endpoints is not statistically significant in the overall population at a two-sided significance level of 0.04, the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.01 level of significance in an analogous manner to the primary analysis in the overall population. If both co-primary endpoints in the overall population are statistically significant at the two-sided 0.04 level, then the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.05 level of significance in an analogous manner to the primary analysis in the overall population. The analysis of secondary and exploratory endpoints will be described in a statistical analysis plan (see appendix) which will be finalized before the first patient is enrolled.

Patient and public involvement

Patients were not involved in the study design. Patients and Patient organizations (in Sweden Barndiabetesfonden) support recruitment through dissemination of information and participation in press conferences. Participating patients and caregivers will be informed about the outcome of the trial via webcast, letter and personal communication on the completion of the trial.

ETHICS AND DISSEMINATION

The trial will be performed in accordance with International Council for Harmonisation (ICH) guidelines, Good Clinical Practice (GCP) and principles of the Helsinki Declaration. The study has been approved by Ethics Committees in Poland (ref number: 124/2021), Netherlands (ref number: R21.089), Sweden (Ref number: 2021-05063), Czech Republic (ref number: EK-1144/21) Germany (ref number: 2021361) and Spain (ref number: 21/2021). Recruitment of participants is planned to start during 2022. Once the trial is completed, results will be published in international peer-reviewed scientific journals and presented at national and international conferences. The main paper will include the primary and secondary outcomes. The manuscript will be submitted to an international peer-reviewed journal, and both positive, negative and inconclusive results will be published. The findings of the trial will be shared with participating sites and presented at national and international conferences. The results will be registered at ClinicalTrials.gov, in EudraCT and will be disseminated to the public.

Contributorship statement

JL conceived the idea and wrote the protocol for the DIAGNODE-1 trial on which the current trial is based on. Thus, the design of DIAGNODE-3 is based on the ideas of JL, with further support from UH, MW and AL. The protocol is written by JL, UH, LE, CN, PFT, MW, AL. JL, UH, LE, CN, PFT, MW, AL, RC and ML have taken part in writing and reviewing the manuscript. All authors have approved the manuscript for publication.

Competing Interest

JL has received unrestricted grants from Diamyd Medical, and honoraria as consultant from Dompé International and Provention Bio. ML has received research grants from Eli Lilly and NovoNordisk and been a consultant or received honoraria from Astra Zeneca, Boehringer Ingelheim, Eli Lilly and NovoNordisk. LE, CN, PFT, MW, AL and UH are all employees of Diamyd Medical. CN, PFT, MW and UH own shares in Diamyd Medical.

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Data sharing statement

No additional data available

Licence statement

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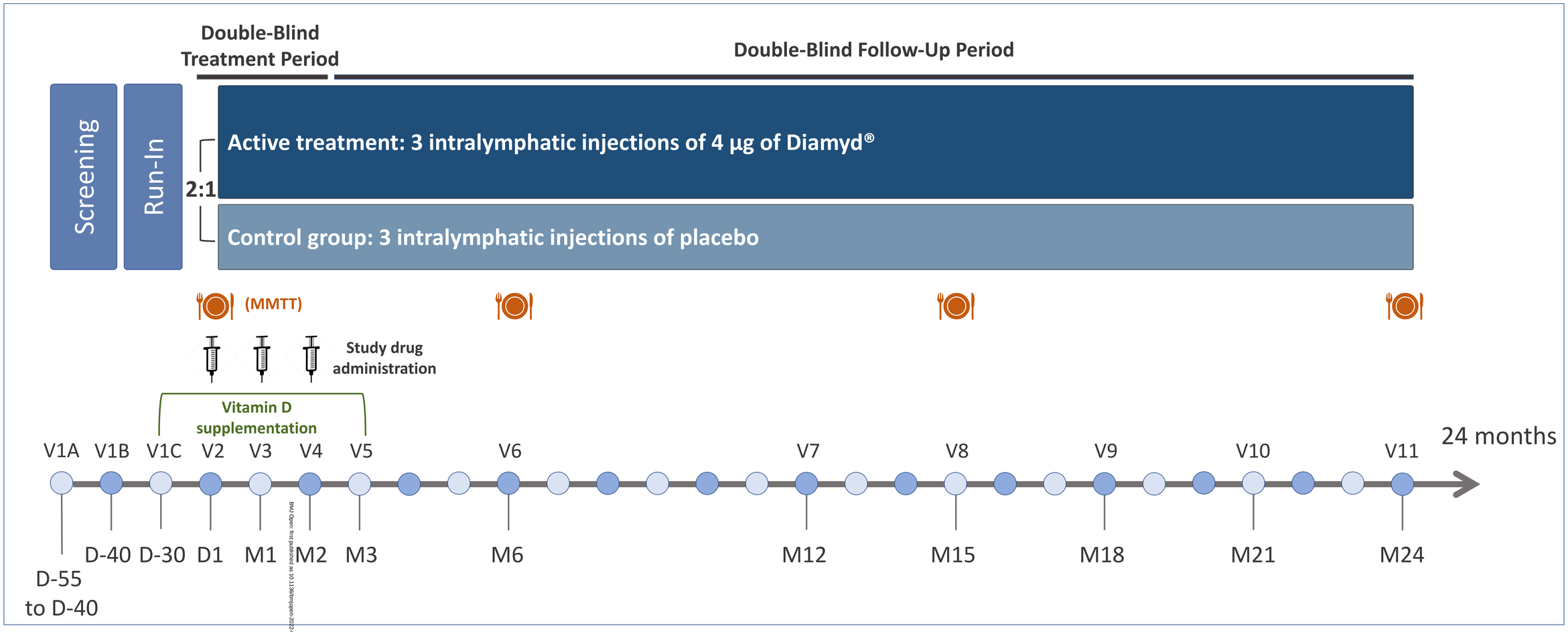
Figure Legend:

Figure 1 - Schematic overview of the study design



Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

A Phase III, Randomized, Double-blind, Placebo-controlled, Multicenter Trial to Evaluate the Efficacy and Safety of rhGAD65 to Preserve Endogenous Beta Cell Function in Adolescents and Adults with Recently Diagnosed Type 1 Diabetes, Carrying the Genetic HLA DR3-DQ2 Haplotype – The DIAGNODE-3 study protocol.

Johnny Ludvigsson[1,2], Linnea Eriksson[3], Christoph Nowak[3,4], Pedro F. Teixeira[3], Martina Widman[3], Anton Lindqvist[3], Rosaura Casas[1], Marcus Lind[5,6,7], Ulf Hannelius[3]

DIAGNODE-3 Statistical Analyses Plan

Populations for analyses

The following analysis sets will be used for the statistical analysis and presentation of data:

- The screened set will consist of all patients who were screened for participation in this study. The screened set will be used for presentation of study disposition of patients.
- The randomized set will consist of all patients who were randomized.
- The safety set (SAF) will consist of all randomized patients who received at least one injection. Patients will be analyzed according to treatment received rather than randomized. If a patient received more than one randomized treatment, they will be analyzed and included in summaries according to the treatment they received the most. Patients receiving no study treatment will be excluded, as will patients who have no post-dose safety assessments. Safety analyses will be based on the SAF.

- The FAS will consist of all randomized patients who have received at least one dose of study medication, a baseline measurement and have at least one post-baseline assessment for any efficacy endpoint. The FAS is the primary analysis dataset, and will be used for all primary, secondary and exploratory efficacy endpoints. Patients in the FAS will contribute to the analysis "as randomized".
- The per protocol set (PPS) will consist of all patients in the FAS who meet the following criteria:
  - Have no important protocol deviations;
  - Completed the treatment phase (Month 24) for the primary end point (i.e., did not discontinue from the trial early);
  - Received all injections of study drug.

C-peptide

The null hypothesis (H_0) is that there is no difference versus the alternative hypothesis (H_1) that there is a difference in the geometric mean ratio (GMR) between the Diamyd-treated group and the placebo-treated group. The null and alternative hypotheses testing can be formalized as follows:

$$H_0: \text{GMR (Diamyd/placebo)} = 1 \quad \text{vs.} \quad H_1: \text{GMR (Diamyd/placebo)} \neq 1$$

where GMR (Diamyd/placebo) is the back-transformed least square mean (LSM) ratio in the relative change from baseline in $AUC_{\text{mean 0-120 min}}$.

Change from baseline will be analyzed using a Restricted Maximum Likelihood-based repeated measures approach (MMRM). The model for analysis will include fixed, categorical effects of treatment, stratification variables, visit, treatment-by-visit interaction, as well as the continuous, fixed covariate of log-transformed baseline C-peptide $AUC_{\text{mean 0-120 min}}$ during an MMTT and the interaction between baseline C-peptide-by-visit, and the fixed continuous covariate of baseline age. Patient identification number will be included as a categorical random effect. An unstructured covariance matrix will be assumed. If this analysis fails to converge, compound symmetry will be tested. The (co)variance structure converging to the best fit, as determined by Akaike's information criterion will be used as the primary analysis. The Kenward-Roger approximation will be used to estimate denominator degrees of freedom.

LSM estimates and 95% CIs will be back-transformed from the natural log scale to the original scale and presented together with nominal p-values. Back-transformed estimates of the treatment difference will provide an estimate of the (Diamyd/placebo)-ratio in the relative change from baseline in $AUC_{\text{mean 0-120 min}}$. A ratio of e.g., 0.8 will mean that the change from baseline to Month 24 in C-peptide level was 20% smaller for Diamyd than for placebo at Month 24.

The primary efficacy analyses will be repeated using the PPS.

HbA1c

If the null hypothesis for the first primary endpoint C-peptide is rejected, then the second primary endpoint HbA1c will be tested. The null hypothesis (H_0) is that there is no difference versus the alternative hypothesis (H_1) that there is a difference in the mean change from baseline to EoS in HbA1c between the Diamyd-treated group and the placebo-treated group. The null and alternative hypotheses testing can be formalized as follows:

$$H_0: \mu\text{Diamyd} = \mu\text{Placebo} \quad \text{vs.} \quad H_1: \mu\text{Diamyd} \neq \mu\text{Placebo}$$

where μ is mean change from baseline to EoS in HbA1c.

If the null hypothesis for the first primary endpoint is not rejected then the hierarchical testing in the overall DR3-DQ2-positive population stops; p-values for the second primary endpoint will be regarded as exploratory.

Change from baseline will be analyzed with the MMRM model and subject to the sensitivity analyses described in Section 0.

HLA DR4-DQ8-negative Subgroup

If either of the co-primary endpoints is not statistically significant in the overall population at a two-sided significance level of 0.04, the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.01 level of significance in an analogous manner to the primary analysis in the overall population. If both co-primary endpoints in the overall population are statistically significant at the two-sided 0.04 level, then the co-primary endpoints in the HLA DR4-DQ8-negative subgroup will be tested sequentially at the 0.05 level of significance in an analogous manner to the primary analysis in the overall population.

Statistical Analyses of Other Endpoints

Analyses of Secondary Endpoints

The following secondary efficacy endpoint will be analyzed with a similar MMRM model as the primary efficacy endpoint (details on log-transformations will be provided in the SAP):

- Change in time in glycemic target range 3.9 to 10 mmol/L (70 to 180 mg/dL) [evaluated from CGM data] between baseline and 24 months.

A specific section of the SAP will lay out in detail the processing and statistical analysis of the raw CGM device data.

The following secondary efficacy endpoint will be analyzed using the Cochran/Mantel-Haenszel Test stratified by the stratification variables; 95% CIs will be calculated according to the Clopper-Pearson method:

- Proportion of patients with IDAA1c ≤9 (partial remission) at 24 months.

The following secondary efficacy endpoints will be assessed using Poisson regression, including stratification variables; rate ratios with 95% CI and p-value will be given:

- Number of episodes per patient of severe hypoglycemia between baseline and 24 months.
- Number of episodes per patient of DKA between baseline and 24 months.

Analyses of Exploratory Endpoints

The following exploratory endpoint variables will be analyzed with a similar MMRM model as the primary efficacy endpoint (details on log-transformations will be provided in the SAP):

- Change from baseline to Month 24 in IDAA1c.
- Change from baseline to Month 24 in exogenous insulin requirements based on total number of units of insulin per kilogram body weight per day.
- Change in time in severe hypoglycemic range <3.0 mmol/L (50 mg/dL) [evaluated from CGM data] between baseline and Month 24.
- Change in time in hypoglycemic range 3.0 to 3.8 mmol/L (50 to 69 mg/dL) [evaluated from CGM data] between baseline and Month 24.
- Change in glycemic variability as measured by %CV [evaluated from CGM data] between baseline and Month 24.
- Change in (fasting, maximal, and stimulated) C-peptide measured at 0, 30, 60, 90, and 120 minutes during MMTT at Month 24.
- Change in serum GAD65A titers between baseline and Month 24.
- Change in QoL evaluated by PRO measures (PedsQL), family impact, generic and diabetes module with parent proxy between baseline and Month 24.
- Change from baseline to Month 24 in BMI.

The following exploratory endpoint will be assessed using Poisson regression, including stratification variables; rate ratios with 95% CI and p-value will be given:

- Number of episodes per patient of mild/moderate hypoglycemia between baseline and Month 24.

The following exploratory endpoints will be analyzed using the Cochran/Mantel-Haenszel Test stratified by the stratification variables; 95% CIs will be calculated according to the Clopper-Pearson method:

- Proportion of patients with a stimulated 90 min C-peptide level above 0.2 nmol/L (0.6 ng/mL) at Month 24.
- Proportion of patients with new onset hyperthyroidism, hypothyroidism, and celiac disease.
- Proportion of patients with increase or decrease in medication usage for treatment of hyperthyroidism and hypothyroidism in those with such disorders at baseline.
- Proportion of patients who change insulin delivery method during the study (MDI/CSII/semi/closed loop system).

Analysis of Safety and Immunological Endpoints

The safety endpoints will be evaluated based on the SAF

Immunological endpoints will be summarized descriptively, including p-values from non-parametric statistical tests (details to be provided in the SAP).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	#3	Date and version identifier	N/A
Funding	#4	Sources and types of financial, material, and other support	18
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1 & 18
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	18
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	10

Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	5-7
Objectives	#7	Specific objectives or hypotheses	8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	9
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	1, 9-10
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	Table 1 and 2

		individuals who will perform the interventions (eg, surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10, figure 1
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	14
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	13-14
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9-10
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8, 15
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended	Figure 1, Table 3

		(see Figure)	
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	9, 15
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	10
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to	10

		interventions	
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	10
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13-14
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	16
Data management	#19	Plans for data entry, coding, security, and storage,	N/A

		including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	15-16
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	15-16, appendix
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	15-16, Appendix
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A

Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	Abstract, 18
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9, Table 3
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in	N/A

		order to protect confidentiality before, during, and after the trial	
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	18
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	9
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	N/A

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A